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Apparent amino acid availability from feedstuffs in extruded diets for rainbow trout *Oncorhynchus mykiss*

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Abstract

Apparent amino acid availability coefficients of several typical and novel feed ingredients were determined in rainbow trout using extruded diets and the faecal stripping technique. The ingredients were tested included five fish meals, three terrestrial animal by-products, five plant protein concentrates, four plant meals, and seven low-protein plant ingredients. Amino acid availability from the fish meals was relatively high ranging from 90 to 101%. Lower coefficients overall were observed for Menhaden fish meal FAQ when compared to the other fish meals. No differences in apparent amino acid availability were detected among the animal by-products. Within the plant concentrate group, rice protein concentrate and barley protein concentrate exhibited generally lower amino acid availabilities compared to other concentrates tested. Among the plant meals, only the availabilities of histidine, valine, isoleucine and lysine in flaxseed meal were lower than those of soybean meal. Apparent amino acid availabilities among the low-protein plant products were variable and significantly different.

KEY WORDS: amino acid, availability, Rainbow trout

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Introduction

Fish meals have historically been used in carnivorous fish feeds due to their high protein content, well-balanced amino acid profile and ability to support rapid growth with high feed efficiency. Although variability in protein quality has been reported among fish meals, increased efforts to control the quality of raw ingredients and improve processing have reduced variability and ensured a consistently high quality protein product. Plant protein sources have become more prominent ingredients in diets for aquatic animals, although inherent problems culminating in reduced fish growth and health continue to plague efforts to increase dietary inclusion levels. The increased interest in plant proteins as primary ingredients for fish feeds is due to the rapid rise in fish meal costs and concerns over the environmental sustainability of fishmeal in aquatic animal feeds (Subasinghe & Phillips 2007; Tacon & Nates 2007). Moreover, increasing knowledge of the nutritional requirements of finfish has allowed further reductions in the level of fish meal in aquafeeds without substantial performance lags. However, one of the limitations to increased utilization of plant products in fish feeds has been protein concentrations in price competitive ingredients that are too low to completely replace fish meals because carnivorous fish diets usually contain in excess of 400 g kg^{-1} dietary protein. Recent developments in ingredient processing have resulted in an increased number of novel protein concentrates from plants that may become price competitive replacements for fish meal in carnivorous fish feeds.

Increasing utilization of alternative ingredients in place of fish meals for aquafeeds is a major focus of the aquaculture industry. Gatlin *et al.* (2007) recently reviewed the use of sustainable plant products in aquafeeds and highlighted the need for better characterization of ingredients. Information on potential limiting nutrients in fishmeal replacements that has not been previously addressed during formulation (Gaylord *et al.* 2006; Lunger *et al.* 2007; Aksnes *et al.* 2008), and limitations of various anti-nutrients in plant-based ingredients (Tacon 1997) have recently been published. Glencross *et al.* (2007) also reviewed strategies generally employed in the evaluation of ingredients for aquaculture feeds with one of the five key evaluation points being measurement of nutrient digestibility, and information on nutrient digestibility from an array of common and novel ingredients has been forthcoming for carnivorous fish (Gaylord *et al*. 2004, 2008a,b; Glencross *et al*. 2004; Rawles *et al*. 2006).

One limitation of the information previously published has been a paucity of data on amino acid availability, especially in extruded feeds. Determining amino acid availabilities with extruded diets has often been prohibitively expensive due to the cost of extruding experimental diets for a large number of ingredients. Extrusion is the predominant method for the production of salmonid feeds. Extruded feeds not only allow better visualization of feeding activity and decrease feed waste, but they also improve starch digestibility through gelatinization, thereby improving digestible energy (DE) in an ingredient, especially for carnivorous fish that are not efficient at utilizing high-carbohydrate food items (Stone 2003). On the other hand, extrusion processing also has been shown to reduce protein digestibility (Cheng & Hardy 2003) and specific amino acid availability (Sorensen *et al*. 2002; Singh *et al*. 2007).

To date, only limited information is available on the digestibility of protein and availability of amino acids from ingredients in extruded compound diets for rainbow trout. Therefore, the current experiment was conducted to assess apparent availability of amino acids for rainbow trout from a widely disparate group of traditional as well as novel feed ingredients, especially plant proteins, that are either currently included or that may have potential for inclusion in trout feeds.

Table 1 Composition of the reference diet

Ingredient	g kg^{-1} (dry-weight basis)
Menhaden fish meal, Special SelectTM [1]	550
Wheat flour[2]	344.9
Menhaden fish oil[2]	80
Vitamin C[3]	3
Choline Cl 50%[1]	5
Vitamin premix[1]	6
Trace mineral[1]	1
Yttrium oxide[4]	0.1
Chromic oxide[4]	10
Analysed composition	**g kg^{-1} (dry-weight basis)**
Crude protein	462.6
Phosphorus	18.2
Energy	5097

[1] Omega Protein Corp., Hammond, LA, USA.
[2] Nelson & Sons Inc., Murray, UT, USA.
[3] Vitamin C as Rovimix® Stay-C® 35, DSM Nutritional Products, Basel, Switzerland.
[4] Sigma-Aldrich Company, St. Louis, MO, USA.

Materials and methods

Ingredients and diets

The 24 ingredients tested were grouped into five classes based on their origin and utility in aquafeed formulations. The five ingredient classes were fish meals, terrestrial animal by-products, plant protein concentrates, plant protein meals with >250 g kg^{-1} crude protein, or plant meals used as a source of carbohydrate that have protein concentrations <250 g kg^{-1} crude protein. The indirect method for determining apparent availability of 10 essential amino acids in the test ingredients was used with yttrium oxide as the inert maker (Cho *et al*. 1982; Bureau *et al*. 1999). Chromic oxide also was included as an alternative inert marker for potential comparisons. In brief, a complete reference diet was formulated that met or exceeded all known nutritional requirements for rainbow trout (Table 1). Test diets were then formulated for each of the test ingredients (Table 2) using a 70 : 30 ratio (dry-weight basis) of reference diet to test ingredient. All ingredients (Table 1) were ground using an air-swept pulverizer (model 18-H; Jacobson, Minneapolis, MN, USA) to a particle size of < 250 μm. All diets were manufactured using a twin-screw cooking extruder (DNDL-44; Buhler AG, Uzwil, Switzerland) with six barrel sections an 18-s transit time through the barrel. The material was not steam conditioned prior to extrusion and water was added to barrel section 2 at a rate of 4.93 gph. The temperature of barrel sections 2, 3, 4, 5 and 6 were maintained at 35, 118, 126, 126 and 124 °C, respectively. The die plate was water cooled to an average temperature of 56 °C. Pressure at the die head varied from 200 to 320 psi, depending on test ingredient. The 3.0 mm pellets were then dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102 °C with a 10-min cooling period. Final moisture levels were <10%. All oil was included in the mix rather than top-coated.

Fish and sample collection

Rainbow trout (*Oncorhynchus mykiss*), Housecreek strain, were obtained from the College of Southern Idaho (Twin Falls, ID, USA) and stocked at a rate of 50, 250 g fish per 140-L fibreglass tank. Water temperature was maintained at 15 °C during the experiment using flow-through spring water. Lighting was set on a 14 : 10 h diurnal cycle. Diets were fed to triplicate groups of fish, and were replicated over

Table 2 Composition of five classes of ingredients fed to rainbow trout to determine the availability of essential amino acids

Ingredient	Class	Moisture (g kg^{-1})	CP (g kg^{-1} dry weight)	His	Thr	Arg	Tyr	Val	Met	Phe	Ile	Leu	Lys
Anchovy fish meal[1]	1	73.0	730.5	19.0	31.5	51.5	25.7	37.6	20.6	31.3	33.5	54.4	56.0
Menhaden fish meal–FAQ[1]	1	85.4	734.7	15.3	28.2	58.5	22.9	32.8	18.9	27.2	28.2	48.1	49.9
Menhaden fish meal, Special Select™[1]	1	68.5	705.0	18.7	29.8	51.3	23.3	35.4	20.1	28.2	30.7	51.5	52.8
Sardine fish meal[1]	1	58.1	718.5	13.2	29.5	55.2	22.1	32.1	19.2	26.9	27.6	46.9	47.5
Sardine fish meal–Mexican[1]	1	52.8	694.5	24.4	30.2	50.0	24.6	36.2	19.9	28.8	30.7	51.9	54.0
Blood meal–poultry, spray dried[2]	2	105.1	964.5	54.1	53.1	53.6	34.3	82.7	15.3	73.9	10.4	124.0	88.6
Feather meal[1]	2	54.7	873.8	5.7	39.5	71.9	27.7	67.1	5.2	45.2	40.5	68.5	12.9
Poultry by-product, Petfood grade[2]	2	39.8	663.9	13.3	26.2	55.1	21.7	31.3	13.7	26.7	26.0	46.3	39.2
Barely protein concentrate 25[3]	3	89.7	304.6	5.9	9.4	21.2	10.4	13.8	3.8	15.9	11.0	19.5	10.0
Corn gluten meal[1]	3	89.6	701.9	13.4	25.2	27.3	39.1	32.4	15.2	45.9	29.1	113.2	12.5
Rice protein concentrate 70[4]	3	75.8	847.4	18.7	30.6	89.5	47.8	49.2	19.2	47.2	36.1	69.9	27.5
Soy protein concentrate[5]	3	56.8	721.6	18.1	30.5	67.5	29.5	35.3	10.2	38.4	33.9	56.5	42.8
Wheat gluten meal[2]	3	65.9	832.7	17.4	23.0	35.9	28.7	35.8	15.5	42.9	32.5	58.7	13.0
Canola meal[1]	4	83.2	490.7	11.6	20.2	33.9	14.6	23.7	7.9	18.9	18.5	32.0	23.3
Cotton seed meal[6]	4	114.2	467.9	12.6	15.8	69.4	15.9	21.7	5.9	26.5	15.7	28.1	18.7
Flaxseed meal[1]	4	61.3	275.4	5.3	10.0	31.9	7.7	13.4	3.9	12.6	11.6	15.5	9.1
Soybean meal, solvent extr., dehulled[1]	4	82.4	538.9	13.1	21.1	48.9	21.1	25.3	6.5	27.5	24.4	39.9	30.0
Barley–Waxbar[7]	5	84.8	215.1	4.6	7.1	12.4	7.9	10.8	3.3	12.0	8.3	17.0	6.8
Corn, whole[1]	5	113.6	106.9	2.5	3.4	5.0	3.0	4.6	1.5	4.3	3.2	10.4	2.9
Rice bran[6]	5	75.7	173.9	4.1	6.2	16.4	6.3	9.1	2.4	7.4	5.9	11.4	6.8
Wheat flour[1]	5	131.2	131.9	2.2	3.1	4.7	3.5	4.6	1.6	5.3	4.0	7.4	2.2
Wheat middlings[1]	5	111.3	207.4	4.6	5.9	14.1	5.7	8.6	2.0	7.6	6.0	11.3	6.6
Wheat millrun[1]	5	112.5	211.9	4.6	5.8	14.5	5.4	8.6	1.6	7.2	5.9	11.1	6.8
Wheat, whole[1]	5	97.1	165.8	3.2	4.1	8.8	4.6	6.6	2.0	7.1	5.3	9.7	3.8

[1] Rangen Inc., Buhl, ID, USA.
[2] Nelsons and Sons Inc, Murray, UT, USA.
[3] Parrheim Foods, Manatoba, Canada.
[4] A&B Ingredients, Fairfield, NJ, USA.
[5] Profine VF, The Solae Company, Fort Wayne, IN, USA.
[6] Planters Cotton Oil Mill Inc., Pine Bluff, AR, USA.
[7] WestBred LLC., Butte, MT, USA.

time. Diets were randomly assigned to a tank of fish and the fish were fed to apparent satiation twice daily. The fish were fed their respective diets for seven days prior to faecal collection.

Faecal samples were obtained in one collection by manual stripping 16–18 h postprandial. Manual stripping of fish was accomplished by netting and anesthetizing all fish in the tank, followed by gentle drying and then application of pressure to the lower abdominal region to express faecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Fish were replaced with new fish before each diet was randomly assigned to a tank of fish for the second and third replicates of feeding for each diet. Faecal samples from a given tank of fish representing a single replicate were dried overnight at 50 °C and stored at −20 °C until chemical analyses were performed.

Chemical analysis

Dry matter analysis of ingredients, diets and faeces was performed according to standard methods (AOAC 1995). Yttrium was determined in diets and faeces by inductively coupled plasma atomic absorption spectrophotometry (University of Idaho Analytical Laboratory Services, Moscow, ID, USA). Crude protein (N × 6.25) was determined in ingredients, diets and feces by the Dumas method (AOAC 1995) on a Leco TruSpec N nitrogen determinator (LECO Corporation, St Joseph, MI, USA). Ingredient, diet and faecal amino acids were quantified according to Fleming *et al.* (1992) with an 1100 series HPLC (Agilent Technologies, Inc., Palo Alto, CA, USA). Briefly, samples were capped with nitrogen and hydrolysed in 6 M HCl at 110 °C for 16 h (AOAC 1995). All samples were derivatized with

O-phthaldialdehyde (P0532; Sigma-Aldrich Co., St Louis, MO, USA) immediately prior to injection on a 5 μm Agilent Hypersil AA ODS column using an automated injection sequence.

Calculation of apparent nutrient digestibility

Apparent availability coefficients of each amino acid in the test diet and ingredients were calculated according to the following equations (Kleiber 1961; Forster 1999):

$$\mathrm{ADCN_{diet}} = 100 - 100\ \{\%\mathrm{Yt\ in\ diet} \times \%\mathrm{nutrient\ in\ feces}\}/\{\%\mathrm{Yt\ in\ feces} \times \%\mathrm{nutrient\ in\ diet}\}$$

$$\mathrm{ADCN_{ingredient}} = (a + b)\mathrm{ADCN_t} - (a)\mathrm{ADCN_r} \times b^{-1}$$

where $\mathrm{ADCN_{ingredient}}$ = apparent digestibility coefficient of the nutrient in the test ingredient; $\mathrm{ADCN_t}$ = apparent digestibility coefficients of the nutrient in the test diets; $\mathrm{ADCN_r}$ = apparent digestibility coefficients of the nutrient in the reference diet; $a = (1 - p) \times$ nutrient content of the reference diet; $b = p \times$ nutrient content of the test ingredient; p = proportion of test ingredient in the test diet.

Statistical analysis

A one-way ANOVA was conducted of mean apparent availability coefficients of the 10 essential amino acids within each of the five ingredient classes using Proc GLM of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Differences among means (n = three tanks/diet) of individual amino acid availabilities among test ingredients in a particular class were determined using the Tukey–Kramer procedure for pair-wise comparisons (Tukey 1953; Kramer 1956). Treatment effects were considered significant at $P < 0.05$.

Results

Among the five fish meals tested (class 1), both protein digestibility and apparent availabilities of the 10 essential amino acids were high and >89% (Table 3). The digestibility of protein in fair and average quality (FAQ) menhaden fish meal was lower at 86% (Gaylord *et al*. 2008a), and the availabilities of histidine, threonine, arginine, isoleucine, and lysine, differed compared to some of the other tested fish meals. Protein digestibility among the tested animal by-products (class 2) ranged from 85 to 88%, while amino acid availabilities also were relatively high and ranged from 77 to 97%; however, differences in availabilities were only detected for arginine among these ingredients.

Although protein digestibility was relatively high (91–99%) among the plant protein concentrates (class 3), differences in availabilities were more pronounced for all amino acids except threonine. Rice protein concentrate 70 and barley protein concentrate 25 generally produced lower apparent amino acid availability coefficients in rainbow trout than soy protein concentrate, corn gluten meal and wheat gluten meal. Among the tested plant protein meals (class 4), protein digestibility was moderately high and ranged from 74% in flaxseed meal to 89% in soybean meal. Availabilities of essential amino acids in soybean meal also tended to be higher than those in the other ingredients of this class. However, availabilities of essential amino acids in the plant protein meals were all >77%.

In contrast, protein digestibility among the low-protein plant meals tested (class 5) ranged from 57% in Waxbar barley to 85% in whole wheat. Apparent amino acid availabilities also were more variable among these ingredients as evidenced by high SEMs observed in the mean availabilities of arginine, tyrosine, methionine, and lysine among these ingredients. Moreover, differences in amino acid availability among these ingredients were detected for all amino acids except lysine even though lysine availability ranged from 66% (whole wheat) to 90% (wheat middlings).

Discussion

Knowledge of the amino acid availability coefficients for feedstuffs used in rainbow trout and other carnivorous fish diets is increasing as the aquaculture industry moves toward greater reduction of fish meal in feed formulations. A number of research groups have determined apparent amino acid availabilities of feed ingredients for rainbow trout (Yamamoto *et al*. 1997, 1998; Cheng & Hardy 2003b; Thiessen *et al*. 2003, 2004). Yamamoto *et al*. (1997, 1998) determined the apparent and true amino acid availability coefficients from a variety of ingredients for rainbow trout using single test diets with individual ingredients as the sole protein sources. Although single source test diets tend to produce less variable coefficients than those of the current trial which employed compound test diets, Yamamoto *et al*. (1997, 1998) only tested high-protein ingredients in their experiment. Yamamoto *et al*. (1998) also found increased apparent and true amino acid availability from soybean meal and malt protein flour in trout from extrusion processing of single ingredients.

Several differences that exist between the methods employed in the current trial and those of Yamamoto *et al*. (1998) that require consideration. Single-source test diets

Table 3 Apparent availability coefficients (%) of essential amino acids in five classes of ingredients fed to rainbow trout[1]

Ingredient	Class	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Tyr	Val
Anchovy fish meal	1	97	99^{a}	96^{ab}	101^{a}	94	97^{ab}	99	94	97^{a}	95	101^{a}
Menhaden fish meal–FAQ	1	86	92^{b}	92^{b}	90^{c}	94	95^{b}	94	92	91^{b}	92	90^{b}
Menhaden fish meal–Special Select^{TM}	1	90	99^{a}	101^{a}	98^{ab}	99	100^{a}	98	98	100^{a}	98	99^{ab}
Mexican sardine meal	1	89	97^{a}	99^{ab}	94^{bc}	98	99^{ab}	97	96	97^{ab}	96	95^{ab}
Sardine fish meal	1	90	98^{a}	98^{ab}	96^{ab}	99	99^{ab}	97	98	98^{a}	97	97^{ab}
Pooled SEM[2]			0.74	1.51	1.26	2.32	1.09	1.29	2.03	1.17	1.86	1.38
Blood meal–poultry, spray dried	2	88	97^{a}	87	86	87	90	91	89	90	87	81
Feather meal	2	85	92^{b}	88	87	88	91	85	91	88	86	85
Poultry by-product meal, Petfood grade	2	85	90^{b}	78	77	87	89	87	84	82	83	80
Pooled SEM			1.00	2.72	3.78	3.6	2.19	4.53	2.86	3.59	3.47	3.97
Barely protein concentrate 25	3	92	93^{b}	89^{b}	80^{b}	91^{b}	85^{c}	83^{b}	91^{b}	87	89^{b}	89^{a}
Corn gluten meal	3	92	99^{a}	96^{a}	91^{a}	97^{a}	91^{b}	92^{a}	95^{a}	93	95^{a}	94^{a}
Rice protein concentrate 70	3	91	94^{ab}	82^{c}	74^{b}	86^{c}	94^{ab}	69^{c}	86^{c}	85	83^{c}	79^{b}
Soy protein concentrate	3	99	98^{ab}	97^{a}	92^{a}	97^{a}	96^{a}	96^{a}	96^{a}	93	96^{a}	93^{a}
Wheat gluten meal	3	101	97^{ab}	95^{a}	92^{a}	97^{a}	95^{ab}	97^{a}	97^{a}	94	96^{a}	94^{a}
Pooled SEM			1.20	1.21	1.92	0.95	1.1	1.5	0.82	1.92	0.91	1.64
Canola meal	4	79	92	96^{a}	85^{b}	92	88^{ab}	87	90	90	90	87^{b}
Cotton seed meal	4	75	94	93^{ab}	83^{b}	89	84^{ab}	82	90	90	90	87^{b}
Flaxseed meal	4	74	93	85^{b}	80^{b}	92	81^{b}	77	87	84	92	84^{b}
Soybean meal, solvent extr., dehulled	4	89	98	100^{a}	98^{a}	98	93^{a}	90	95	95	95	99^{a}
Pooled SEM			1.62	1.88	2.75	2.33	2.42	3.43	2.83	3.22	3.33	2.61
Barley–Waxbar	5	57	91^{ab}	67^{cd}	61^{b}	79b^{c}	68	64^{ab}	82^{b}	62^{c}	76^{ab}	72^{bc}
Corn, whole	5	65	99^{a}	89^{ab}	86^{a}	94^{a}	73	64^{ab}	71^{b}	73^{bc}	80^{ab}	90^{ab}
Rice bran	5	64	86^{ab}	60^{d}	57^{b}	78^{c}	68	61^{b}	72^{b}	74^{bc}	74^{ab}	71^{c}
Wheat flour	5	82	42^{b}	71^{bcd}	87^{a}	91^{ab}	71	85^{a}	92^{a}	96^{a}	73^{b}	95^{a}
Wheat middlings	5	68	79^{ab}	96^{a}	90^{a}	96^{a}	90	72^{ab}	90^{a}	90^{ab}	90^{ab}	93^{a}
Wheat millrun	5	69	96^{a}	93^{a}	89^{a}	99^{a}	81	68^{ab}	91^{a}	92^{a}	96^{a}	94^{a}
Wheat, whole	5	85	64^{ab}	81^{abc}	92^{a}	90^{ab}	66	84^{a}	90^{a}	94^{a}	82^{ab}	97^{a}
Pooled SEM			8.97	3.96	3.48	2.42	5.98	4.19	2.57	3.26	4.09	3.63

[1] Different superscripts (a,b,c) within a column for an ingredient class indicates significant differences at $P < 0.05$.
[2] Pooled standard error of the mean.

employed by Yamamoto *et al*. (1998) may not have mimicked ingredient-nutrient interactions typical of compound extruded diets. Moreover, not only was the availability data from Yamamoto *et al*. (1998) generated from smaller fish (ca. 14 g) than those stocked in the current trial (ca. 250 g), but the former work employed a faecal settling column compared to the faecal stripping technique used in the current trial. Faecal collection technique can affect availability coefficients as well as comparisons between the experiments (Anderson *et al*. 1995). Although the latter group extruded individual test ingredients, the ingredients were then incorporated into compounded diets that were cold-pelleted. Cold pelleting would limit reactions that normally change amino acid, as well as carbohydrate and lipid, availability to fish during extrusion processing of compounded diets.

Other difficulties also arise in comparing amino acid availabilities across trials as well as species. For example, two factors are the variability of ingredients over time as well as different sources. Ljokjel *et al*. (2000) demonstrated that nitrogen and amino acid digestibility in soybean meal and fish meal decreased in mink when either ingredient was heated up to 150 °C for 30 min. In contrast, Sorensen *et al*. (2002) found no differences in protein digestibility or amino acid availability in rainbow trout when extrusion temperature varied from 100 to 150 °C. Sorensen *et al*. (2002) suggested there may be differences in the susceptibility of mink and trout to damaged amino acids, or that endogenous losses in trout may mask small differences in availability. Yamamoto *et al*. (1998) found differences in apparent protein digestibility and amino acid availabilities that were fish species dependent as well as feed ingredient dependent. For example, amino acid availabilities in corn gluten meal were lower for common carp and red sea bream than for rainbow trout, while minimal differences were detected in the availabilities of amino acids in other feed ingredients tested among the three fish species. Although the same ingredient was fed across species, potential differences in intake may affect the apparent digestibilities between species for an individual ingredient. In the current trial, extrusion temperature was maintained at approximately 127 °C for a relatively short period of time (18 s) to mimic commercial conditions and minimize potential heat-induced amino acid damage.

It is often observed that variation in the digestibility coefficient for a particular nutrient increases as the nutrient content of that ingredient decreases. Evidence of this phenomenon can be observed in the current trial where average SEMs for all amino acids in plant-based ingredients with <250 g kg^{-1} protein were higher (4.26) than those of fish meals (1.46) or the plant protein concentrates (1.32). Although variability was higher among the low-protein plant ingredients relative to other ingredient groups, potential inaccuracies in amino acid availability from these ingredients will have little practical effect on diet formulations due to their limited inclusion levels (<300 g kg^{-1}) and thus low protein/amino acid contribution to the diet.

Another possible factor affecting amino acid availability is the potential for Maillard reaction product formation. The extrusion process employed in the current study (127 °C for 18 s) may limit Maillard product formation when intact starches are included (Cheftal 1986); however potential lysine destruction cannot be overlooked. Although direct comparisons were not made in the current experiment between plant-protein concentrates and their low protein, high-carbohydrate counterparts, there is the potential that lysine availability was reduced in the latter due to the higher potential for reducing sugars and formation of Maillard products. Martinez-Amezcua & Parsons (2007) postulated that ingredient types will vary in their susceptibility to heat-induced damage when they observed higher lysine destruction in corn distillers dried grains with solubles compared to other feed ingredients. Batterham (1992) also points out that heat-damaged amino acids, such as threonine, methionine, tryptophan and lysine, may induce errors in apparent amino acid availability estimates because they are in a form that can be absorbed but can not be utilized for protein synthesis.

When evaluating complete extruded diets, errors may occur in estimation of available lysine. Rutherford & Moughan (2007) suggested that the acid hydrolysis procedure may revert early Maillard reaction products back to lysine giving high total lysine estimates for feed ingredients, but biologically they will be unavailable to the animal for protein synthesis. The use of acid hydrolysis for determination of faecal lysine for digestibility estimates also may skew results on lysine availability by underestimating reactive lysine that is digested and absorbed.

In conclusion, data from the current trial provide some of the information necessary to formulate diets with reduced fish meal content on an available amino acid basis. There are a variety of techniques that have been used to determine amino acid availability, and the approach used in the current trial was chosen to represent practical, commercial type, feeds and conditions.

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References

Aksnes, A., Munheim, H., Toppe, J. & Albrektsen, S. (2008) The effect of dietary hydroxyproline supplementation on salmon (*Salmo salar* L.) fed high plant protein diets. *Aquaculture*, **275,** 242–249.

Anderson, J.S., Lall, S.P., Anderson, D.M. & McNiven, M.A. (1995) Availability of amino acids from various fish meals fed to Atlantic salmon (*Salmo salar*). *Aquaculture*, **138,** 291–301.

AOAC. (1995) *Official Methods of Analysis*. Association of Official Analytical Chemists, Inc., Arlington, VA, USA.

Batterham, E.S. (1992) Availability and utilization of amino acids for growing pigs. *Nutr. Res. Rev.*, **5,** 1–18.

Bureau, D.P., Harris, A.M. & Cho, C.Y. (1999) Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **180,** 345–358.

Cheftal, J.C. (1986) Nutritional effects of extrusion-cooking. *Food. Chem.*, **20,** 263–283.

Cheng, Z.J. & Hardy, R.W. (2003a) Effect of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutr.*, **9,** 77–83.

Cheng, Z.J. & Hardy, R.W. (2003b) Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **218,** 501–514.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982) Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comp. Biochem. Physiol.*, **73B,** 25–41.

Fleming, J., Taylor, T., Miller, C. & Woodward, C. (1992) *Analysis of Complex Mixtures of Amino Acids Using the HP 1050 Modular HPLC*. Application Note 228-212, publication no. 5091-5615E. Agilent Technologies Inc., Palo Alto, CA, USA.

Forster, I. (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquacult. Nutr.*, **5,** 143–145.

Gatlin, D.M. III, Barrows, F. T., Bellis, D. *et al*. (2007) Expanding the utilization of sustainable plant products in aquafeeds – a review. *Aquacult. Res*., **38,** 551–579.

Gaylord, T.G., Rawles, S.D. & Gatlin, D.M. III (2004) Amino acid availability from animal, blended and plant feedstuffs for hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquacult. Nutr*., **10,** 345–352.

Gaylord, T.G., Teague, A.M. & Barrows, F.T. (2006) Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *J. World Aquacult. Soc*., **37,** 509–517.

Gaylord, T.G., Barrows, F.T. & Rawles, S.D. (2008a) Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for tainbow trout, *Oncorhynchus mykiss*. *J. World Aquacult. Soc*., **39,** 827–834.

Gaylord, T.G., Barrows, F.T., Rawles, S.D., Liu, K., Bregitzer, P., Hang, A., Obert, D. & Morris, C. (2008b) Apparent digestibility of nutrients in extruded diets from cultivars of barley and wheat selected for nutritional quality in rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr*. doi: 10.1111/j.1365-2095.2008.00595.x

Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P., Hawkins, W.E., Maas, R. & Sipsas, S. (2004) A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **237,** 333–346.

Glencross, B.D., Booth, M. & Allan, G.L. (2007) A feed is only as good as its ingredients–a review of ingredient evaluation strategies for aquaculture feeds. *Aquacult. Nutr*., **13,** 17–34.

Kleiber, M. (1961) *The Fire of Life: An Introduction to Animal Energetics*. John Wiley and Sons, Inc., New York, New York, USA.

Kramer, C.Y. (1956) Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, **12,** 307–310.

Ljokjel, K., Harstad, O.M. & Skrede, A. (2000) Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. *Ani. Feed Sci. Tech*., **84,** 83–95.

Lunger, A.N., McLean, E., Gaylord, T.G. & Craig, S.R. (2007) Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). *Aquaculture*, **271,** 401–410.

Martinez-Amezcua, C. & Parsons, C.M. (2007) Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. *Poult. Sci*., **86,** 331–337.

Rawles, S.D., Gaylord, T.G. & Gatlin, D.M. III (2006) Digestibility of gross nutrients by sunshine bass *Morone chrysops* × *M. saxatilis* from animal by-products and commercially blended products used as fishmeal replacements. *N. Am. J. Aquaculture*, **68,** 74–80.

Rutherford, S.M. & Moughan, P.J. (2007) Development of a novel bioassay for determining the available lysine contents of foods and feedstuffs. *Nutr. Res. Rev*., **20,** 3–16.

SAS Institute Inc.. (1985) *SAS User*'s Guide: Statistics, Version 5 edn. SAS, Cary, NC, 956 pp.

Singh, S., Wakeling, L. & Gamlath, S. (2007) Retention of essential amino acids during extrusion of protein and reducing sugars. *J. Agric. Food. Chem*., **55,** 8779–8786.

Sorensen, M., Ljokjel, K., Storebakken, T., Shearer, K.D. & Skrede, A. (2002) Apparent digestibility of protein, amino acids and energy in rainbow trout (*Oncorhynchus mykiss*) fed a fish meal based diet extruded at different temperatures. *Aquaculture*, **211,** 215–225.

Stone, D.A.J. (2003) Review: Utilization of dietary carbohydrate by fish. *Rev. Fish. Sci*., **11,** 337–369.

Subasinghe, R.P. & Phillips, M.J. (2007) Aquaculture development and environmental capacity: where are the limits? In: *Global Trade Conference on Aquaculture* (Arthur, R. & Nierentz, J. eds), pp. 109–114, Qingdao, China. Food and Agriculture Organization of The United Nations, Rome, 29–31 May 2007.

Tacon, A.G.J. (1997) Fishmeal replacers: Review of anitnutrients within oilseeds and pulses–A limiting factor for the aquafeed green revolution? *Cashiers Options Mediterraneennes (CHIEAM)*, **22,** 153–182.

Tacon, A.G.J. & Nates, S.F. (2007) Meeting the feed supply challenges of aquaculture. In: *Global Trade Conference on Aquaculture* (Arthur, R. & Nierentz, J., eds) pp. 117–121, Qingdao, China. Food and Agriculture Organization of the United Nations, Rome, 29–31 May 2007.

Thiessen, D.L., Campbell, G.L. & Adelizi, P.D. (2003) Digestibility and growth performance of juvenile rainboe trout (*Oncorhynchus mykiss*) fed pea and canola products. *Aquacult. Nutr*., **9,** 67–75.

Thiessen, D.L., Maenz, D.D., Newkirk, R.W., Classen, H.L. & Drew, M.D. (2004) Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr*., **10,** 379–388.

Tukey, J.W. (1953) The problem of multiple comparisons. Unpublished manuscript. Princeton University, Princeton, NJ, USA.

Yamamoto, T., Ikeda, K., Unuma, T. & Akiyama, T. (1997) Apparent availabilities of amino acids and minerals from several protein sources for fingerling rainbow trout. *Fish. Sci*., **63,** 995–1001.

Yamamoto, T., Akimoto, A., Kishi, S., Unuma, T. & Akiyama, T. (1998) Apparent and true availabilities of amino acids from several protein sources for fingerling rainbow trout, common carp, and red sea bream. *Fish. Sci*., **64,** 448–458.

